DEVELOPMENT OF ALGAL ADSORPTION SYSTEMS FOR ENHANCED METAL RECOVERY FROM MINING PROCESS STREAMS AND TAILINGS

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Final Report

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Dr. R.G.L. McCready

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EXECUTIVE SUMMARY

1. Four algal species, Scenedesmus acutus, Chlorella vulgaris, Chlorella pyrenoidosa and Chlamydomonas reinhardtii were evaluated for their suitability for metal adsorption experiments. Only C. pyrenoidosa and S.acutus were found suitable for further investigation. They produced sustained growth under high-light (16 kilolux) and low-nitrogen (33% of normal Bold's Basal Medium, 25 mg/L) conditions.

2. Algal cultures were pretreated under various conditions of light and nutrient status. Some were incubated with cadmium and clay. The physiological stress introduced by these were expected to stimulate the production of extracellular organic compounds which would provide adsorption sites for metal ions.

3. Algal cultures were employed to adsorb cadmium from a stock solution and copper from the Star Lake Gold Mine effluent. Experiments evaluated the effect of growth and operational parameters such as pretreatment methods, culture age, pH and the clay concentration in the clay-algal complexes.

4. Most of the adsorption took place within four minutes of exposure of the algal culture to the metal solution.

5. Cultures took 24 to 28 days to reach the end of their stationary phase in normal light and nutrient conditions. Cultures showed a sharp peak in their adsorption capacity after 28 days. Older cultures, from 28 to 97 days, showed sustained ability to adsorb cadmium and copper.

6. As the cultures grew older they became less sensitive to the variations in the pH of the adsorption experiments.

7. Addition of clay to algae to increase the adsorption was more successful at some culture ages than others. Clay-algal complexes were more efficient in producing adsorption sites than algae incubated with cadmium or clay.

8. Older Chlorella pyrenoidosa cultures were far superior to older Scenedesmus acutus cultures in adsorbing cadmium and copper. The effect of pH on S.acutus was less significant than on C.pyrenoidosa.

9. Forming clay-algal complexes at optimum culture ages and clay concentrations produced significant increases in metal adsorption. Complexes with S.acutus and pure clay were similar in their response. Both resulted in decreased metal uptake [mg/g] when clay content in the complex was increased. C. pyrenoidosa when complexed, increased its adsorption at higher clay content.

10. Chlorella cultures pretreated in low-N and high-light conditions, pure clay and all pretreated Scenedesmus cultures produced a decrease in per cent adsorption $[M_a/M_p].10^2$ as the metal loading $[M_p/A_w]$ increased. However, other Chlorella cultures produced higher adsorption at higher levels of metal loading.

11. All clay complexes with Chlorella cultures excepting those pretreated in Low-N and high-light conditions increased the adsorption per unit weight of the adsorbent as the metal loading increased. The slope of the power-law dependence was 1.3. For the other complexes including those with Scenedesmus and pure clay the slope ranged from 0.15 to 0.89.

All these experimental runs related to copper adsorption from the Star Lake Gold Mine effluent.

12. Concentration factors for copper reached as high as 5868 and the highest copper uptake [mg/g] observed was 98.5 mg/g.

13. Copper could be desorbed uniformly at all pH values from algae. But copper desorbed 100% at pH values of 5 and 11 from a clay-algal complex containing 73% of clay in the total adsorbent. From a complex containing 47% of clay the maximum desorption was only 60% at pH11.

Desorption appeared to be related to the observed Concentration Factor and the clay content. Pure algal cultures desorbed uniformly at all pH values. Higher clay contents brought about a desorption characteristically similar to clay.

14. Clay-algal complexes can be very efficient for adsorbing copper and cadmium from mining effluents.

The observed high [mg/g] values for copper ranging from 70 to 98.5 present a strong case for exploring the algal adsorption systems for mining effluents.

RAPPORT

1. Quarre espéces d'algues, Scenedesmus acutus, Chlorella vulgaris, Chlorella pyrenoidosa et Chlamydomonas reinhardtii ont été évaluées pour leur compatibilité lors d'expériences de l'adsorption de métaux. Seulement C.pyrenoidosa et S.acutus ont été jugées utiles en vue de recherces ultérieures. Elles croissent de façon soutenue sous des conditions de fortes intensités lumineuses (16 kilo lux) et pauvre en azote (33% de la normale du milieu de culture de base Bold, 25 mg/L).

2. Les cultures d'algues ont été prétraitées sous plusieurs conditions de lumiéres et de nutriments. Quelques unes ont été incubées en présence de cadmium et d'argile. Le stress physiologique induits par ceux-ci avait pour but de stimuler la production de composés organiques extracellulaires permettant ainsi l'adsorption d'ions métalliques.

3. Les cultures d'algues ont été employées pour adsorper le cadmium d'une solution de stock et du cuivre provenant de l'effluent du Star Lake Gold Mine. Les expériences ont évalué la croissance et les paramétres expérimentaux tels les méthods de prétraitement, le niveau d'âge de la culture, les concentration du pH et de l'argile dans les complexes d'algues en milieu argileux.

4. La majorité de l'adsorption s'est déroulée en dedans de 4 minutes durant lesquelles les cultures d'algues étaient exposées â la solution métallique.

5. Les cultures ont mis de 24 à 28 jours avant d'atteindre la fin de leur phase stationnaire en conditions

de lumiére normale et de nutriments. Les cultures ont présenté un sommet aigu de leur capacité d'adsorption aprés 28 jours. Les cultures plus âgées, celles de 28 à 97 jours, ont montré une capacité soutenue à l'adsorption du cadmium et du cuivre.

6. Devenant plus âgées, les cultures ont été moins sensibles aux variations du pH des expériences d'adsorption.

7. L'addition d'argile aux algues comme moyen d'accroissement de l'adsorption a eu plus de succés chez certains niveaux d'âge de culture que chez d'autres. Les complexes d'algues en milieu argileux étaient supérieurs en production de sites d'adsorption que les algues incubées avec le cadmium ou l'argile.

8. Les cultures de Chlorella pyrenoidosa plus âgées étaient de beaucoup supérieures aux cultures de Scendesmus acutus en ce qui a trait à l'adsorption du cadmium et du cuivre. L'effet du pH sur le S. acutus était moins significatif que sur le C.pyrenoidosa.

9. La formation de complexes d'algues en milieu argileux aux niveaux d'âge de culture et de concentrations d'argile optimum a produit des accroissement significatifs dans l'adsorption de métal. Les complexes de S.acutus et d'argile pure ont eu des résponses similaires. Les deux ont produit une diminution de l'adsorption du métal quand l'argile contenue dans le complexe était augmentée. Une fois le complexe C.pyrenoidosa formé, il a augmenté son adsorption dans un milieu plus élevé en argile.

10. Les cultures de Chlorella prétraitées dans des conditions lumineuses élevées et pauvre en azote ainsi qu en

présence d'argile pure et toutes les cultures de Scenedesmus ont produit une diminution de quelque pourcent d'adsorption $[M_a/M_p].10^2$ alors que la concentration $[M_p/A_w]$ de métal augmentait. Cependant, certaines cultures de Chlorella ont produit une plus grande adsorption en présence de concentration supérieure de métaux.

11. Tous les complexes d'argile des cultures de Chlorella àl'exception de celles prétraitées dans des conditions lumineuses élevées et pauvre en azote, ont augmenté l'adsorption par unité de poids de l'adsorption en méme temps que la concentration du métal augmentait. La courbe de dépendance du "power-law" était de 1,3. Pour les autres complexes incluant ceux avec du Scenedemus et de l'argile pure, la pente s'est échelonée entre 0,15 et 0,89.

Tous ces essais expérimentaux se rapportent à l'adsorption du cuivre provenant de l'effluent du Star Lake Gold Mine.

12. Les facteurs de concentration du cuivre ont atteint jusqu à 5868 et la plus haute adsorption de cuivre observée a été de 98,5 mg/g.

13. Le cuivre pourrait étre désorbé uniformént à tous les niveaux de pH d'algues. Mais le cuivre s'est désorbé à 100% lorsque le niveau du pH était situé entre 5 et 11, cuivre provenant d'un complexe d'algues en milieu argileux qui contient 73% d'argile dans l'adsorbant total. Dans un complexe contenant 47% d'argile, la désorption maximale fut seulement de 60% à un niveau de pH 11.

La désorption semble étre relieé au facteur de concentration et au contenu d'argile. Les cultures d'algues pures se sont désorbées uniformément à tous les niveaux de pH. Les contenus en argile plus élevés ont apporté à peu prés une désorption charactéristique semblable à l'argile.

14. Les complexes d'algues en milieu argileux peuvent étre trés efficaces pour adsorber le cuivre et le cadmium provenant d'effluents miniers. Les hautes valeurs de [mg/g] observées pour le cuivre s'échelonnant entre 70 et 98,5 représentent un excellent exemple pour l'exploration des systémes d'adsorption d'algues dans les effluents miniers.

DEVELOPMENT OF ALGAL ADSORPTION SYSTEMS FOR ENHANCED METAL RECOVERY FROM MINING PROCESS STREAMS AND TAILINGS

INTRODUCTION:

There have been significant developments in recent years in the application of biotechnological concepts to the processes encountered in the mining industry. Specific metabolic pathways utilized by certain bacteria have been successfully employed to leach metals from ore bodies in a variety of operational modes. Leaching techniques introduced by these bacterial processes have made improved product recovery possible. Recent research efforts into the adsorption, accumulation and precipitation of metals by different types of biological systems have opened up some new and exciting possibilities.

Algal and fungal biomass have been shown to adsorb and metabolize specific metals and toxic compounds from process streams under a wide range of physical and chemical environments (Galun, et al, 1983., Sober et al, 1986). Algae, particularly the green and blue-green species, accumulate large quantities of metals such as Zn, Cu, Pb, Cd, As, Ag, Au, Ni and compounds such as organochlorides, cyanides and PCBs from aqueous solutions without experiencing any deleterious effects. In fact, the bioaccumulation factor, the ratio of the metal concentration in the algal cell to the metal concentration in water, can reach as high as a million. Data from field studies indicate that several species of algae growing in polluted waters contain as much as 190 ppm of arsenic (Hutchinson,1975).

Metal precipitation systems using algal biomass have several distinct advantages which make them preferable to bacterial or chemical processes. There are a number of algal species which lend themselves for mass cultivation and whose growth kinetics is easily controlled. Algal systems can be

designed to selectively remove specific metals or metal complexes to function as a general precipitation mechanism. The rate of adsorption of metals increases with the metal concentration in the medium. However, these organisms have the unique ability to fix their own nitrogen and obtain energy from sunlight and carbon dioxide. Hence they can function effectively to remove metals and chemical compounds from the medium even at low substrate concentrations. This feature gives them an added advantage over some of the bacterial processes which require minimum substrate concentrations to perform effectively (Kobayashi and Rittman, 1982).

Algae can bring about photochemical reactions in the wastewater which can be effectively targeted to degrade or transform specific metals, metal complexes or chemical compounds. Algae produce extra-cellular products such as lipids, polysaccharides and other organic compounds which can bind metals very effectively as part of their normal metabolism or under specific environmental conditions (Lange,1973 and Vymazal 1986). Polysaccharides and peptides excreted by algae produce enormous adsorption sites for metals and the rate of adsorption can be quite significant in terms of quantities adsorbed and the rapidity of adsorption. Chlorella, a green alga, can complete the adsorption process in 15 seconds removing DDT from water. Algae can remove metals and chemicals from continuous wastewater streams and in the case of DDT the following equation describes the removal process (Butler, 1978).

$$C_t = C_o[\ 1 - e^{-Qt/\phi}]$$

where C_t is the concentration of the adsorbed material in the algal cell, C_o is the ambient concentration in water and Q, t and ϕ are the operational variables.

It is also clear from field and controlled studies that algal adsorption increases quantitively with the ambient concentration. Algae remove metals from the medium through three different processes; adsorption to the outside cell wall, transportation and adsorption to the inner cell wall and the metabolic retention within the algal cell (Stokes, 1983). In some experiments these processes contributed to 20%, 30% and 50% of the total removal, respectively (Butler,1978). However, the adsorption processes can be significantly enhanced by the availability of nutrients, particularly phosphorus, and specific culture conditions (Jennet et al, 1982). It is known that arsenic is an eefective chemical substitute for phosphorus and it is found from studies that algae preferentially take up arsenic in the presence of phosphorus (Lockwood, 1976).

The membrane permeability in algal cells for metals can be increased by appropriate changes in pH, ionic environment and the culture conditions (Shumate, 1985). Uptake of metals also depends upon the growth stage of the algae and the presence of colloids.

Pretreatment of algae before contact with the wastewater can signifcantly increase their adsorption rates. Increased photosynthesis alters the internal pH in algal cells which also modifies the membrane permeability. Many algae, including Chlorella, have been shown to produce their own chelating compounds which can bind metal ions and metallic complexes. Synergistic effect of metal ions on the removal of a given metal has also been observed. In some ceses, metal binding proteins of the metallothionein type excreted by the algae have been suggested as possible mechanisms for detoxification.

Accumulation of Pb, Mn and Zn from mining effluents in natural, unmanaged algal growth was, in one instance, found to be 5 mg/g, 4mg/g and 1mg/g (dry weight), respectively (Gale and Wixson, 1979). It is, therefore, encouraging

to explore the practical potential of designed and targeted algal systems to remove significant quantities of heavy metals from mining process streams.

Spisak (1979) proposed an algal-based arsenic removal process where arsenic assimilation would be accomplished in two photosynthetic reactors containing about 1,300 kg of algae in 24,700 kg of aqueous solution. Arsenic laden algae would be harvested from the reactors at a rate of 430 kg/d. The discharged liquid would contain 40 μg/L of arsenic, reduced from the initial 1.0 mg/L concentration.

Recently, the work reported from the New Mexico State University has shown that some species of blue-green algae can adsorb gold and uranium ions from wastewaters from uranium mills. These algae release adsorbed gold in preference to whatever other metals they have adsorbed. Gold was, in these experiments, extracted from algae by desorption by passing a solution of thiourea through the column.

Blue-green algae are often considered prime candidates for metal binding due to their ability to release low-molecular weight compounds of high chelating capacity (Lange, 1973).

System Ecotechnologies Inc. conducted laboratory investigations to determine the suitability and effectiveness of algae for adsorbing metal ions from aqueous solutions. This project formed the initial phase of a larger program to develop algal adsorption systems for enhanced metal recovery from mining process streams and effluents. The second and subsequent phases would conduct pilot and full scale operations based on the data from the preliminary work. The objective of the project was to define various pretreatment and operating conditions to maximize adsorption and desorption of metals from aqueous solutions. Results obtained from bench-scale tests are presented in this report.

MATERIALS AND METHODS:

Algal species were selected based on the previous published work related to metal adsorption, their ability for large growth rates and controllable growth characteristics, their stability under high and low light and nutrient conditions and their ability to produce extracellular products.

Cell cultures for Chlorella vulgaris, Chlorella pyrenoidosa, Chlamydomonas reinhardtii, and Scenedesmus acutus were obtained from the University of Toronto Culture Collection. The first three species are green algae and the last alga is a flagellate. These species can tolerate high nutrient conditions characteristic of polluted environments. Bold's Basal Medium (BBM) was used as the normal nutrient solution and the nutrient composition of this medium is given in the Appendix. Growth tables were specially designed and fabricated to provide adjustable illumination for various pretreatment runs. High-intensity, Durotest fluorescent lamps were used to produce a maximum usable light intensity of 20 kilolux or 391 E per m^2 per second. The light intensity was adjusted to the desired levels by altering the lamp height. Cultures were grown in erlenmeyer flasks with continuous mixing in automatic and adjustable shakers.

Several batches of stable cultures were established to determine the growth characteristics and maximum cell densities. Cells were counted almost on a daily basis using a microscope and a Sedgwick-Rafter device. Optical densities were concurrently measured using the transmission or absorbance at 435nm, 530nm and 660nm with a Turner spectrophotometer. Normally, calibration curves involving the correlation between the optical densities at about 660nm and cell counts have been employed to develop growth curves for algal cultures. However, the absorbance at this wavelength is primarily due to the scattering losses by the cells and

does not contain any information about the physiological activity of the cells. On the other hand, the absorbance at 435nm is contributed by both the chlorophyll absorption and the scattering loss. A difference between the optical densities or transmissions at these two wavelengths provides valuable information on the concentration of physiologically active pigments in the algal cells. In a number of instances where stable cultures were allowed to continue beyond their normal stationary phase the cell counts included a large number of senescing cells with very little chlorophyll or other pigments. It is important to obtain some information about the physiological state of the cells during the time when they are subjected to adsorption experiments. The difference between the transmissions at 660nm and 435nm varied with the concentration of the photosynthetically active pigments in the cells. Fig.3 shows the data obtained for Chlorella pyrenoidosa Batch III.

It is evident from published literature (for example, Fogg 1965, Watt 1966, Watt and Fogg,1966) that extracellular secretions from the algal cells provide adsorption sites for metals and metallic complexes. Further, the normal matabolic uptake of metals during the exponential phase of cell growth is followed by adsorptive metal uptake in the stationary or senescent phase. This latter uptake mechanism is attributed to the presence of polysaccharides and other organic compounds which can be elicited from the cells by drastically altering the growth conditions such as the light intensity and nitrogen availability. The primary thrust of the experimental runs has been to define the effectiveness of using post-stationary phase cultures under low-nitrogen and high-light conditions.

The experimental plan consisted of determining the metal uptake as a function of growth and operating parameters. The growth parameters included light intensity,

nutrient status and the incubation of cell cultures with cadmium and clay. The experimental operating parameters included culture age, pH, exposure or mixing time, clay--algae complexing and successive adsorption runs.

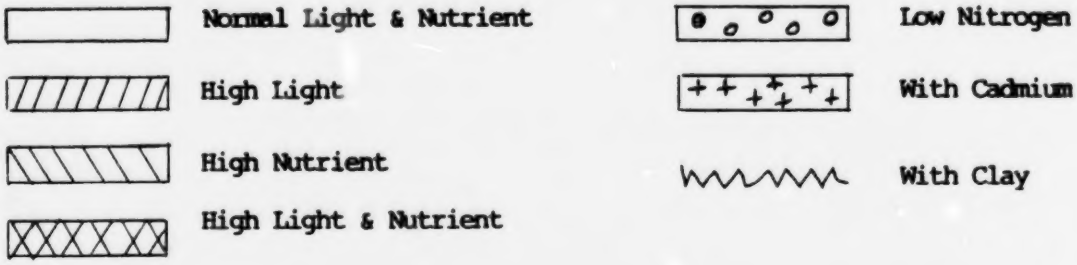
Initially, the experimental runs were performed with cadmium solutions of concentrations ranging from 10.0 mg/L to 115 mg/L. These runs permitted an understanding of the growth parameters as they related to the metal uptake under various pH and culture age conditions. The next set of experimental runs used a mixture of cadmium, copper and iron of varying concentrations. In the third stage of experimental runs, mining effluent from the Star Lake Gold Mine at Star Lake, Saskatchewan was used primarily for the removal of copper.

CANDIDATE SPECIES:

The limited resources available for the project did not permit the evaluation of more than two species of algae and a decision had to be made to choose the candidate species. As indicated before, the effectiveness of an algal species to adsorb significant quantities of metal ions depends upon the presence of extracellular organic compounds. These are produced as a response to high-light and low-nitrogen conditions. Therefore, the choice was made on the basis of the survival of the algal species during prolonged exposure to such extenuating growing conditions. The four algal species Chlorella vulgaris, Chlorella pyrenoidosa, Chlamydomonas reinhardtii and Scenedesmus acutus were grown under high light and their viability was evaluated from their cell counts. Only Scenedesmus acutus and Chlorella pyrenoidosa showed good sustainable growth and further experimental runs were conducted only with these two species.

Stable cultures were established under various pretreatment conditions which are given in Tables 1 to 3.

Table. 1 Culture Status and Growth Conditions



BATCH	CULTURE TREATMENT	START & TERMINATION
SA; CP; CV; CR; I Batch		Dec.11.87 - Jan.8.88
SA; CP; CV; CR; II Batch		Dec.21.87 - Jan.15.88
SA; CP; CV; CR; III Batch		Jan.6.88 - Feb.8.88
SA; CP; IV Batch		Jan.28.88 - Feb.10.88
SA; CP; PT I		Jan.30.88 -
SA; CP; PT II		Feb.5.88 -
SA; CP; V Batch		Feb.10.88 - Mar.2.88
CP; VI Batch		Feb.12.88 - Mar.17.88
SA; VI Batch		Feb.12.88 -
CP; VII Batch		Feb.12.88 - Mar.17.88
SA; VII Batch		Feb.12.88 - May.3.88

(Table 1. Continued)

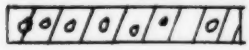
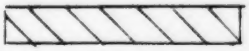
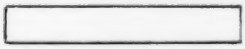
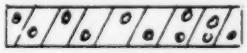
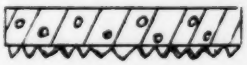
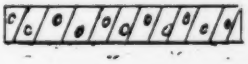
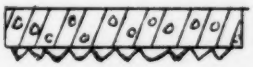
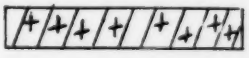
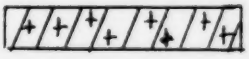
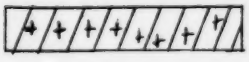
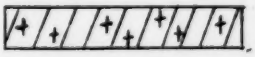
BATCH	CULTURE TREATMENT	START & TERMINATION
CP; SA; PT III		Feb.22.88 - Mar.2.88
CP; PT IV		Mar.17.88 -
CP; VIII Batch		Mar.17.88 - Mar.23.88
CP; IX Batch A,B,C		Mar.23.88 -
CP; PT V & VI		Mar.21.88 - Apr.3.88 Mar.22.88 - Apr.3.88
SA; PT VII		Mar.25.88 - Apr.3.88
SA; PT VII+		Mar.25.88 - Apr.3.88
CP; PT VII		Mar.25.88 - Apr.3.88
CP; PT VII+		Mar.25.88 - Apr.3.88
CP; PT VIII		Mar.17.88 - Apr.27.88 Apr.27.88 -
CP; PT IX		May.6.88 -
SA; PT IX		May.6.88 - Jn.8.88
CP; PT X		May.6.88 - Jn.8.88
SA; PT X		May.6.88 - Jly.6.88
CP; PT XI A,B,C,D,E		May.20.88 -
SA; PT XII A,B,C,D,E		May.20.88 -
CP; PT XIII		May 30.88 -
CP; PT XV		June.26.88 -

Table 2 . Pretreatment Schedules for Algal Cultures

BATCH	HIGH LIGHT	NORMAL LIGHT	HIGH NUTRIENT	LOW N	NORMAL NUTRIENT	WITH Cd	WITH Clay
CP VI		*	*				
CPPT VIII	*	*	*				
CPPT XV	*				*		♯
CPPT VI	*		*				*
CPPT VII	*			*			
CPPT VII+	*			*			*
CPPT IX	*				*		
CPPT IX	*				*		
CPPT X	*				*	*	
CPPT XIA	*				*		♯
CPPT XI B-E	*				*	*	
CPPT XIII	*				*	*	* ♯

Explanation:

HIGH LIGHT : 16 kilolux = 312.5 μ E $m^{-2}sec^{-1}$

NORMAL LIGHT: 5 kilolux = 97.7 μE $m^{-2}sec^{-1}$

NORMAL NUTRIENT: Standard BOLD'S BASAL MEDIUM (See Appendix)

HIGH NUTRIENT: THREE times the Standard Bold's Basal Medium

LOW - N: Nitrogen reduced by a factor of 1000 to 25 mg/L in the Standard Medium.

WITH Cd: Cadmium added to the culture at the start of the culture, at 0.5 mg/L

WITH CLAY: Bentonite Clay added to the culture at the start of the culture 1.87 g/L.

CPPT VIII: Culture was started in normal light and high nutrient . After 41 days (March 17 to April 27) the culture was transferred to high light.

♯: These samples were used in adsorption experiments with clay added at various concentrations to prepare clay-algal complexes.

Table. 3 Pretreatment Schedules for Algal Cultures

BATCH	HIGH LIGHT	NORMAL LIGHT	HIGH NUTRIENT	LOW N	NORMAL NUTRIENT	WITH Cd	WITH CLAY
SA VI	*	*	*				
SA VII	*	*	*				
SAPT VII	*			*			
SAPT VII+	*			*			*
SAPT IX	*				*		*
SAPT X	*				*	*	*
SAPT XI A	*				*		*

SA VI : Culture started Feb 12,1988 under normal light and transferred to high light after 10 days.

SA VII: Culture started Feb 12,1988 under normal light and transferred to high light after 41 days.

Algal dry weights were measured on two occasions (Table.4) and for all computations the values obtained on June 29 were used. These related to the samples from continuous cultures.

Table. 4 Algal Dry Weight Measurements

Date	Culture	Treatment	Cell Count mL^{-1}	Density g/L	Dry Weight $g/10^6$ cells
March 6, '88	Chlorella pyrenoidosa	Batch CP VII 22 days High Nutrient Normal Light	42.0×10^6	0.730	17.4
March 6, '88	Scenedesmus acutus	Batch SA V Normal Nutrient Normal Light	14.3×10^6	0.968	67.7
June 29,'88	Chlorella pyrenoidosa	Continuous Culture Normal Nutrient Normal Light	107×10^6	1.239	11.6
June 29,'88	Scenedesmus acutus	Continuous Culture Normal Nutrient Normal Light	33.0×10^6	1.132	34.3

Aliquots of algae were filtered through 0.45 μ membrane filter and resuspended in the metal solution and mixed for 4 minutes. The mixture was then filtered through 0.45μ membrane filter and the filtrate and the filter were both analysed for the metal. The mixing was done in a rotary shaker at 100 RPM.

STABLE ALGAL CULTURES:

Several batches of algal cultures were developed with normal light intensity, low-nitrogen and normal nutrient conditions so as to familiarize with the species and their growing patterns. Chlorella pyrenoidosa and Scenedesmus acutus were superior to other species in terms of relative growth rate, population density and cell viability under high light (16 kilo lux) conditions. These cultures reached the final stages of stationary phase in about 28 days. Figs. 1-5 show the growth curves for batches II and III. The difference in light transmission at 660nm and 435nm is also plotted along with the cell counts to show the decline in the active pigments in the cells towards the end of the exponential phase.

The algal growth patterns can be described by the following equation,

$$N_t = N_o e^{kt}$$

where N_t is the population at time 't', N_0 is the population at zero time and k is the relative growth constant. A mean doubling time can be defined for the culture which is the time required to double the population and is given by

$$T = \frac{0.6932}{k}$$

T is given in days.

Table. 5 shows the values for k and T for Scenedesmus acutus and Chlorella pyrenoidosa, with some values for the other two algal species.

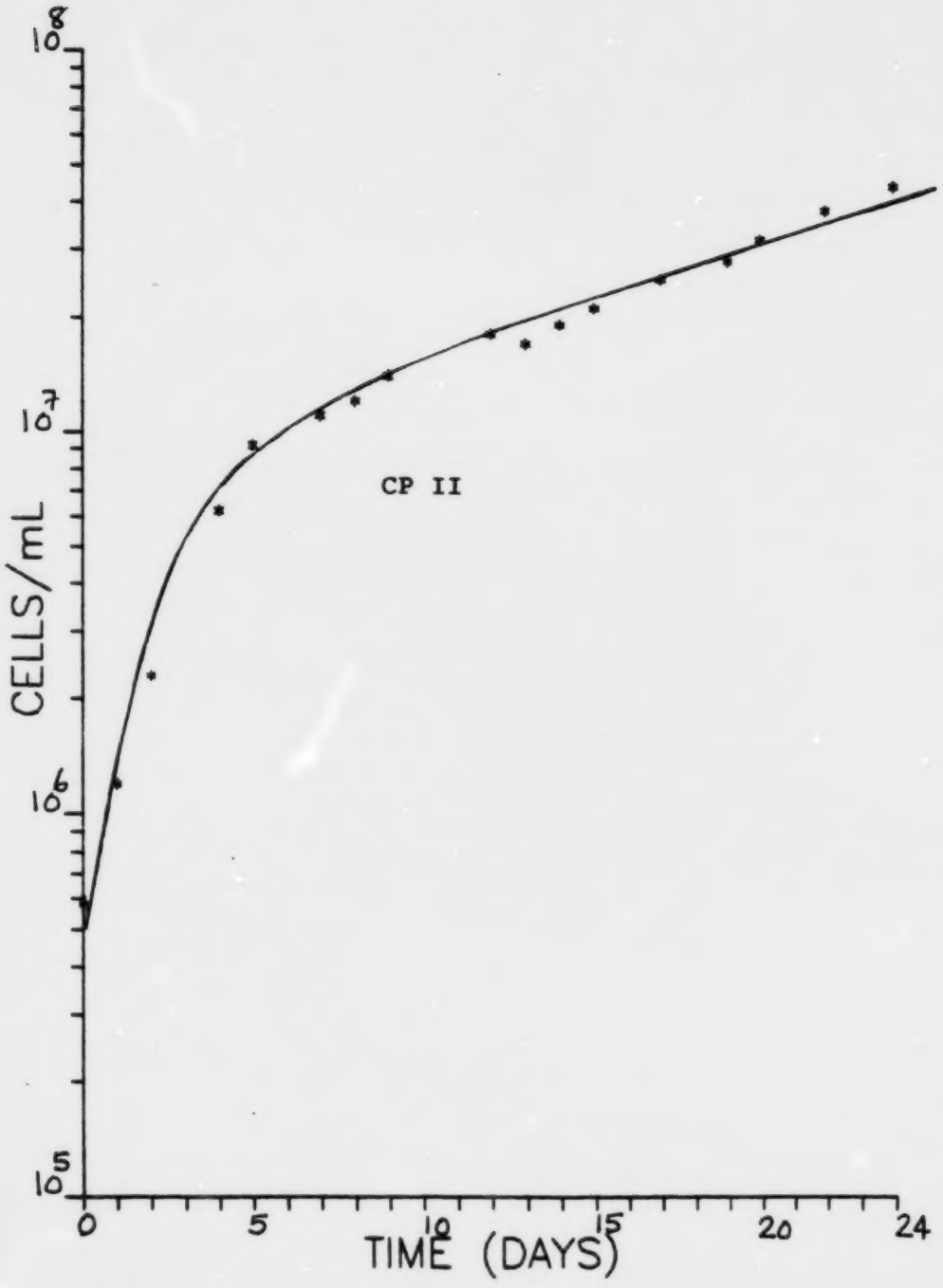


Fig.1 Growth of Chlorella pyrenoidosa in normal light and normal medium (BBM)

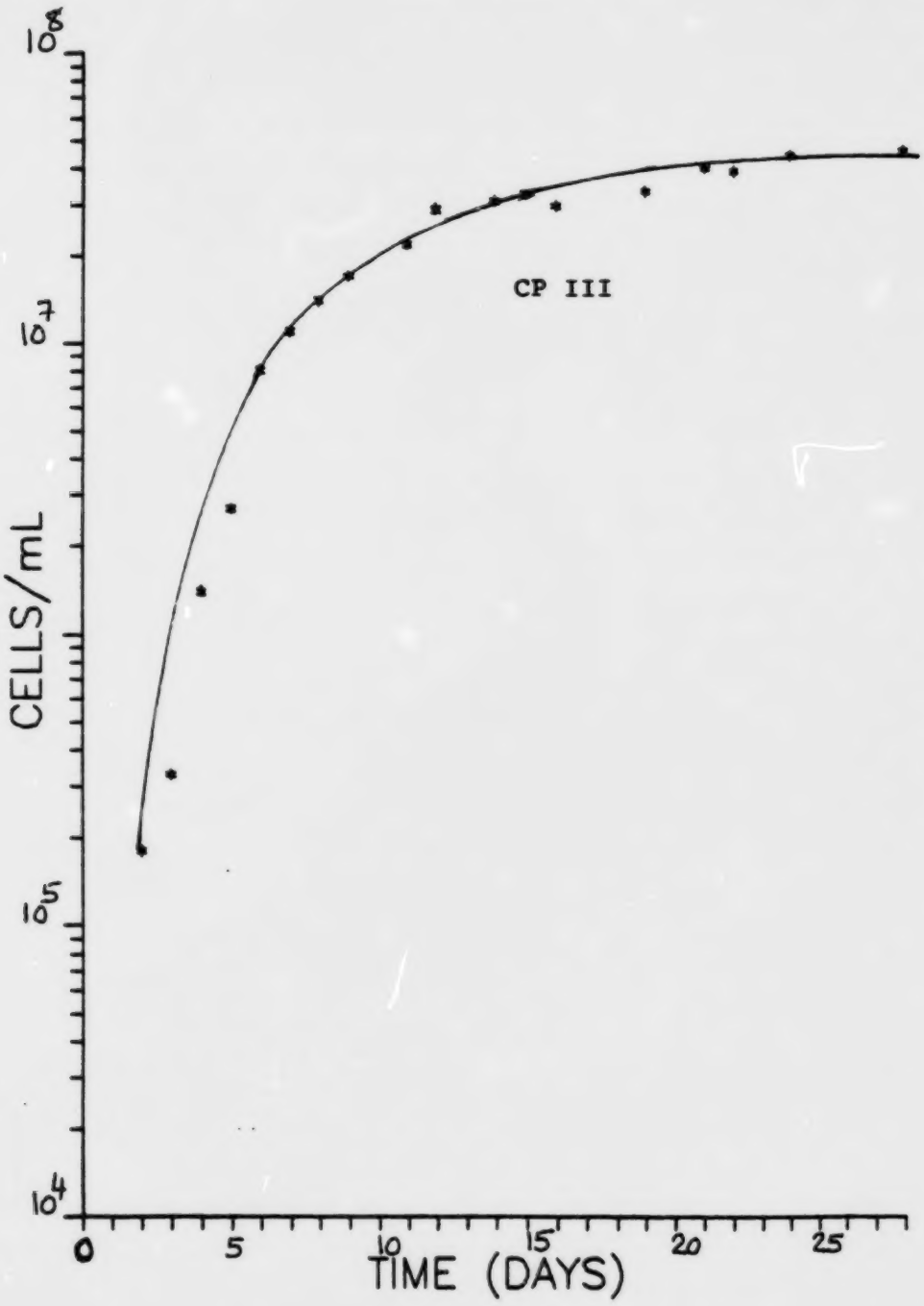


Fig.2 Growth of Chlorella pyrenoidosa normal light and normal medium (BBM).

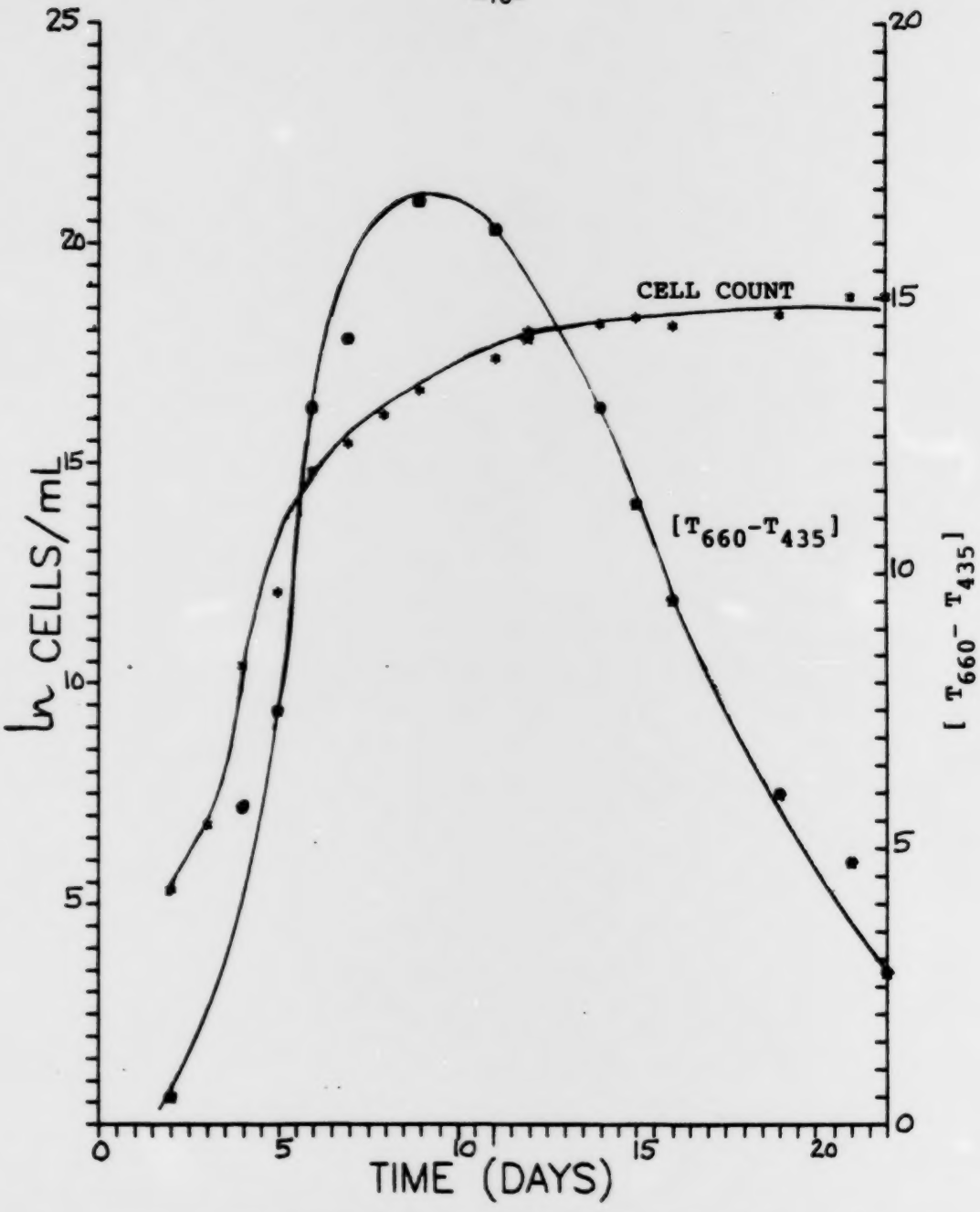


Fig.3 Cell Counts and the Difference in Light Transmission at 660nm and 435nm for Chlorella pyrenoidosa Batch III

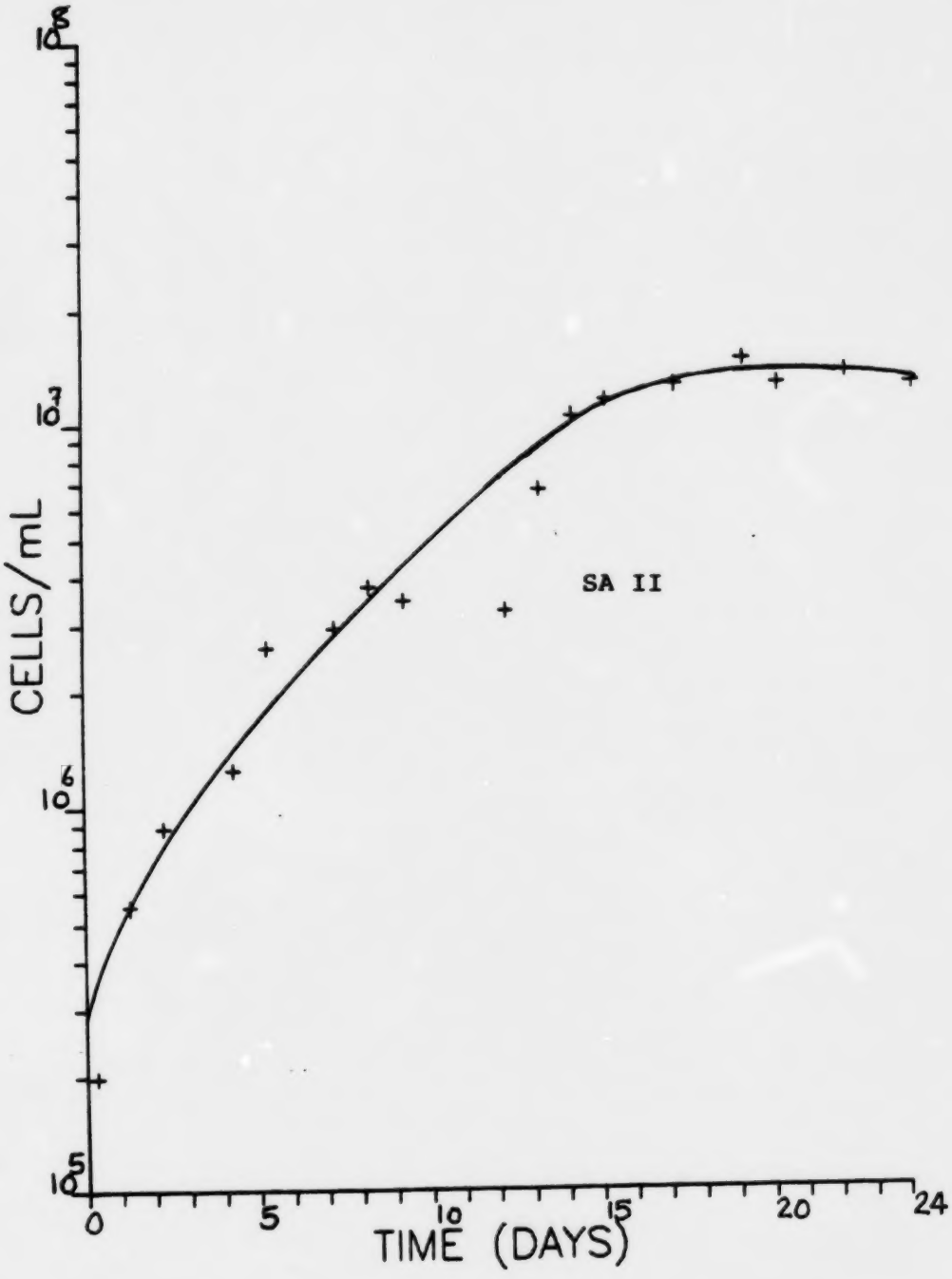


Fig 4 Growth of Scenedesmus acutus in normal light and normal medium (BBM).

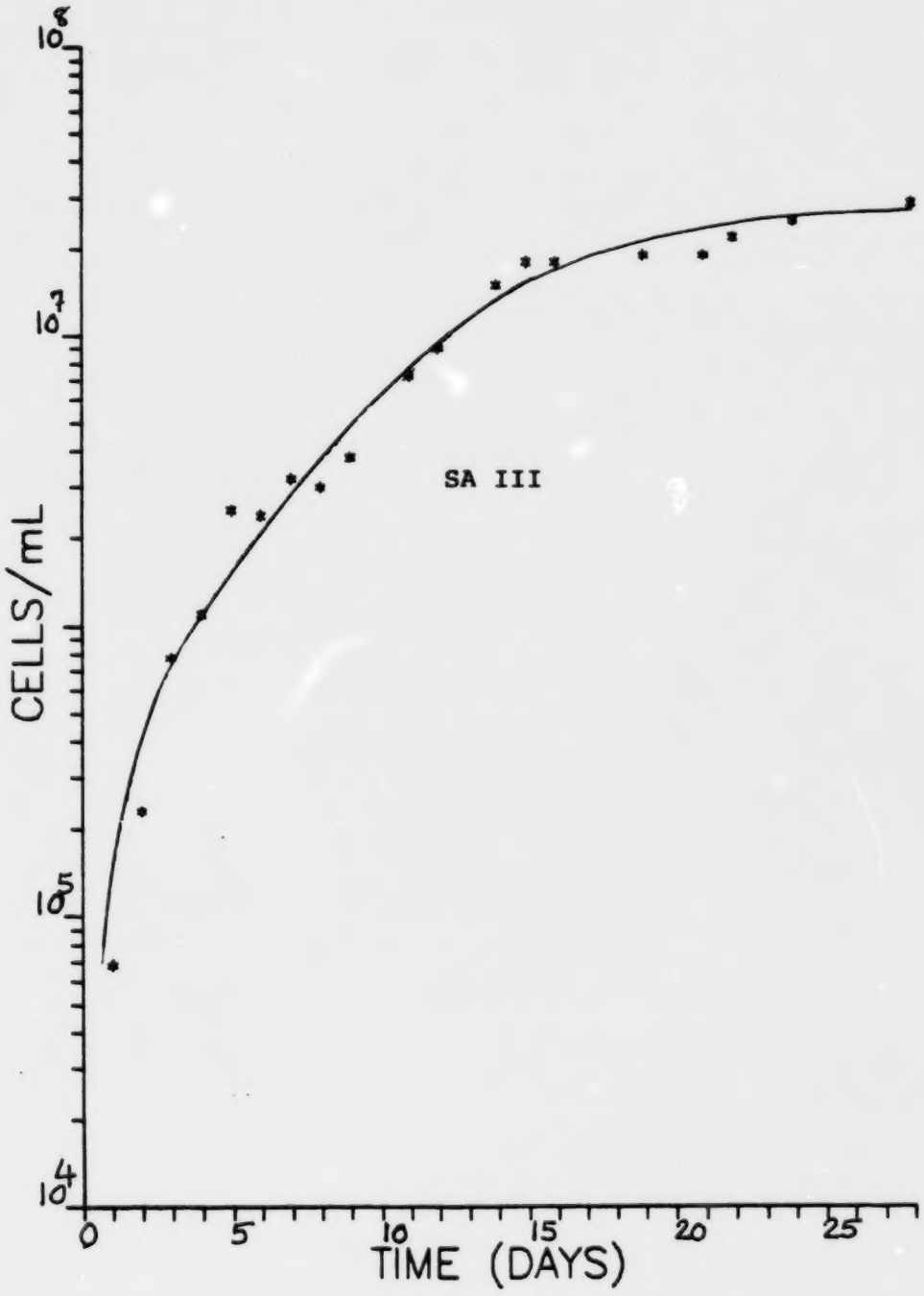


Fig. 5 Growth of Scenedesmus acutus in normal light and normal medium (BBM).

Table.5 Growth Kinetics of Algal Cultures

Scenedesmus acutus

Batch	N_o	k	T
SA II	6.5×10^5	0.16	4.3
SA III	5.1×10^5	0.19	3.7
SA IV	1.6×10^6	0.11	6.3
SA V	2.7×10^5	0.22	3.2
SA VI	3.2×10^5	0.13	5.3
SA VII	1.3×10^6	0.11	6.3
SAPT III	3.3×10^6	0.03	23.1

Chlorella pyrenoidosa

Batch	N_o	k	T
CP II	2.2×10^6	0.14	5.0
CP III	1.3×10^6	0.21	3.3
CP IV	2.0×10^6	0.17	4.1
CP V	5.2×10^5	0.25	2.8
CP VI	1.3×10^6	0.11	6.3
CP VII	2.5×10^6	0.14	5.0

Chlorella vulgaris

Batch	N_o	k	T
CV II	1.2×10^6	0.12	5.8
CV III	2.5×10^5	0.21	3.3

Chlamydomonas reinhardtii

Batch	N_o	k	T
CR II	3.9×10^5	0.12	5.8
CR III	2.8×10^5	0.16	4.3

RESULTS

EXPOSURE TIME:

Aliquots of algal cultures were filtered (0.45 micron membrane filters) and resuspended in metal solution and mixed. Samples were taken at various time intervals and filtered for analysis. In most cases both the filter and the filtrate were analysed and the values checked against each other. Most of the adsorption took place within four minutes, removing 92% of the initial concentration. All experimental runs were conducted with four-minute mixing.

Table. 6 Effect of Mixing Time on Metal Adsorption

Time Interval Mins	Algal Mass mg	Metal Removed mg	Metal Uptake mg/g	% Removal
4	5.54	0.030	5.4	91.6
20	5.54	0.032	5.8	96.0
60	5.54	0.029	5.2	89.0
90	5.54	0.031	5.6	92.5
150	5.54	0.037	6.7	94.5
240	5.54	0.031	5.6	05.1

CULTURE AGE:

Since most of the adsorption is essentially due to many of the organic compounds released by the algae during the post-exponential phase, the culture age is recognized as a very important parameter to evaluate. Algal responses were compared to determine the effect of culture age on specific operating and growth conditions such as low-

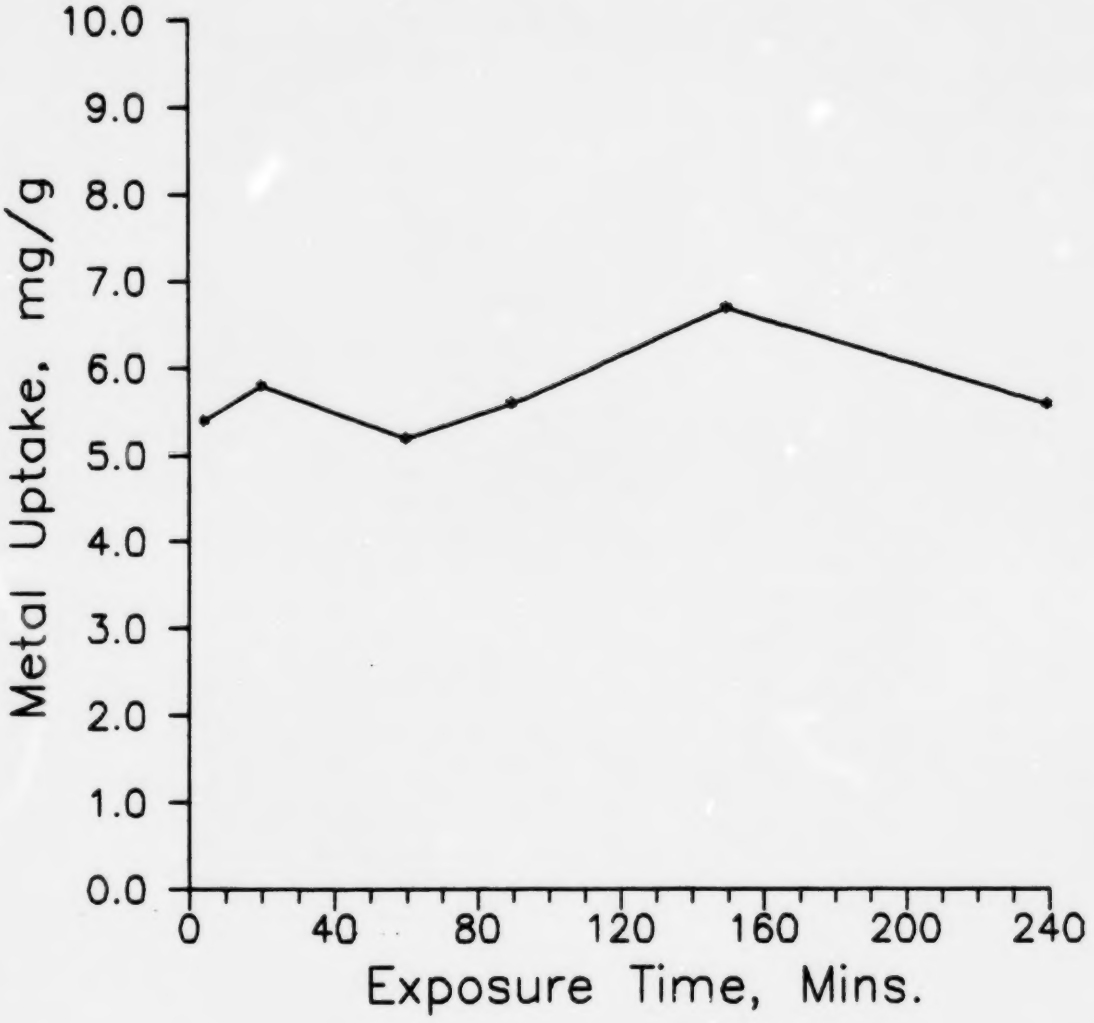


Fig. 6 Cadmium Adsorption for Various Exposure Times

nitrogen status, clay addition, response to pH and the species. Metal uptake increased sharply when the cultures were 28 days old. Algae grown in nitrogen-deficient medium showed a consistently higher uptake than the cultures exposed to normal nutrient medium. The 28-day peak was also higher for the low-nitrogen algae. Algae incubated with clay during their growth showed a lower adsorption than the algae without clay incubation.

Copper adsorption by the culture CPPT XIII without clay reached a high value of 89 mg/g at the culture age of 56 days (Fig.8). There was a distinct difference between Scenedesmus acutus and Chlorella pyrenoidosa in their response to culture age. S.acutus exposed to low-N status with or without clay incubation continued to increase the metal adsorption for 39 days, whereas the culture exposed to high nutrient status (SA VIII) decreased its metal uptake as the culture grew older. The cultures SAPT VII+ and SAPT VII could not be continued beyond 40 days as the culture became non-viable due to senescence. Figs. 8 and 9 show the data obtained.

Incubation with clay did not appear to have any beneficial effect on the algal metal uptake. The peaking effect observed in Chlorella at 28 days was absent in Scenedesmus cultures, implying the possibility that the latter alga does not secrete organic compounds in its post-exponential phase.

ALGAL RESPONSE TO pH:

Adsorption is a pH-dependent process and will have important impact on the economics of metal stripping operations. The response of CP VI and CPPT VIII are compared in Fig.10. Both were pure algal cultures without clay or cadmium contamination. The 27-day CP VI culture showed a considerable variation with pH, with the metal uptake reducing to zero at pH 11. On the other hand,

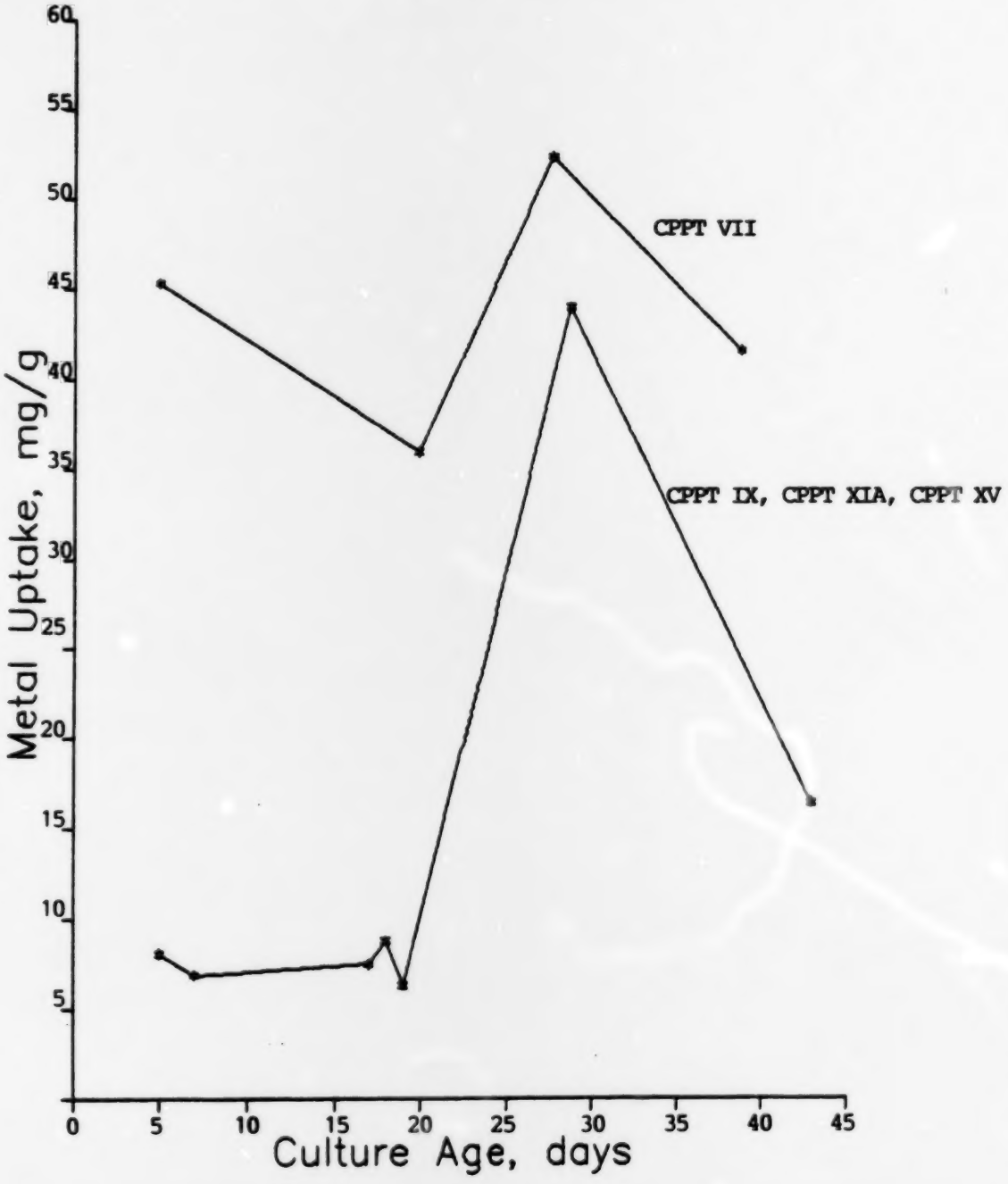


Fig.7 Copper Adsorption from Star Lake Gold Mine Effluent by Pretreated Algal Cultures; CPPT VII (Low-Nitrogen and High Light) and CPPT IX, CPPT XIA, CPPT XV (Normal-Nitrogen and High-Light)

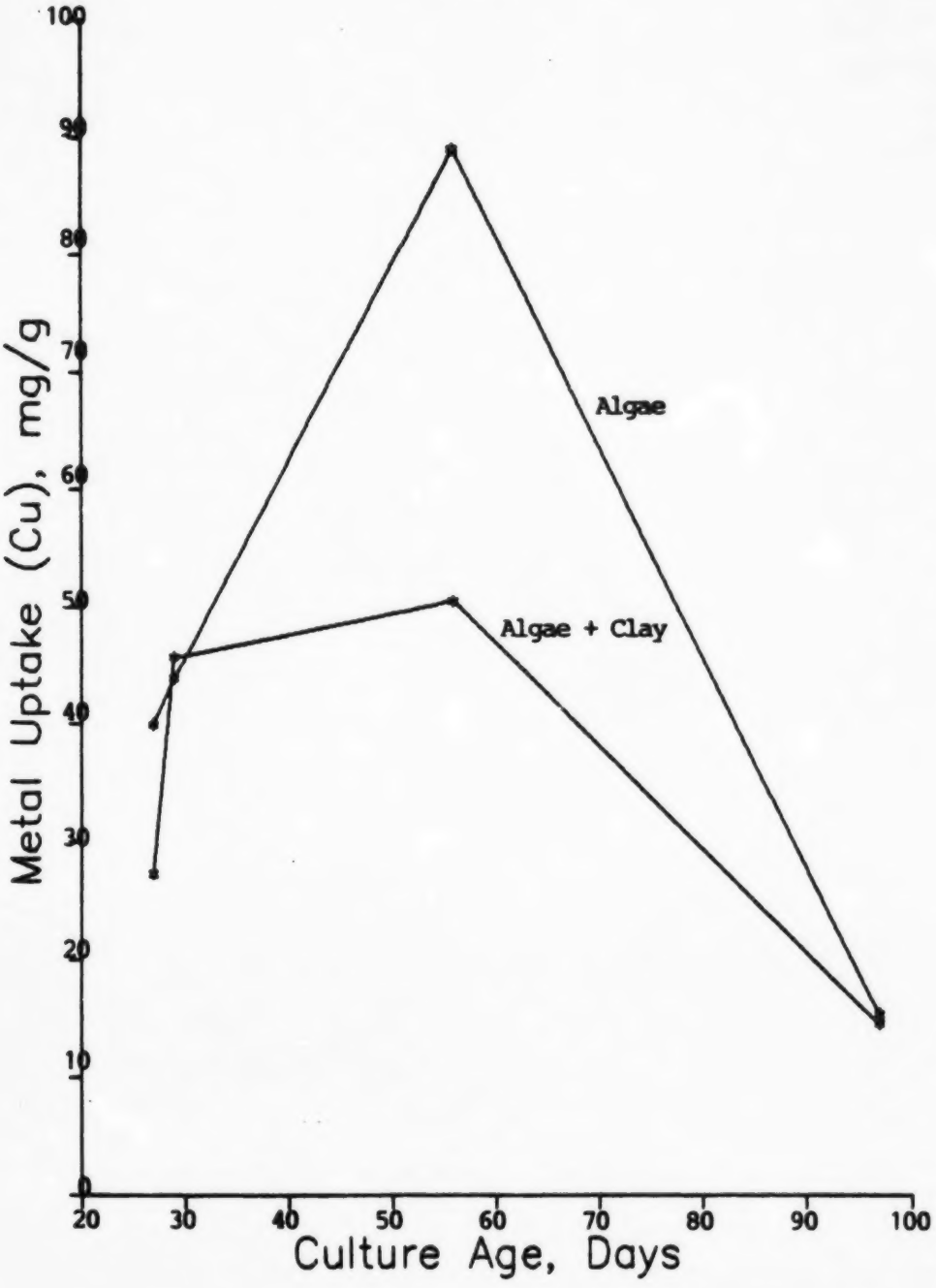


Fig.8. Copper Adsorption from Star Lake Gold Mine Effluent by Pretreated Algal Cultures; CPPT XIA, CPPTXV, CPPT XIII and CPPT VIII.

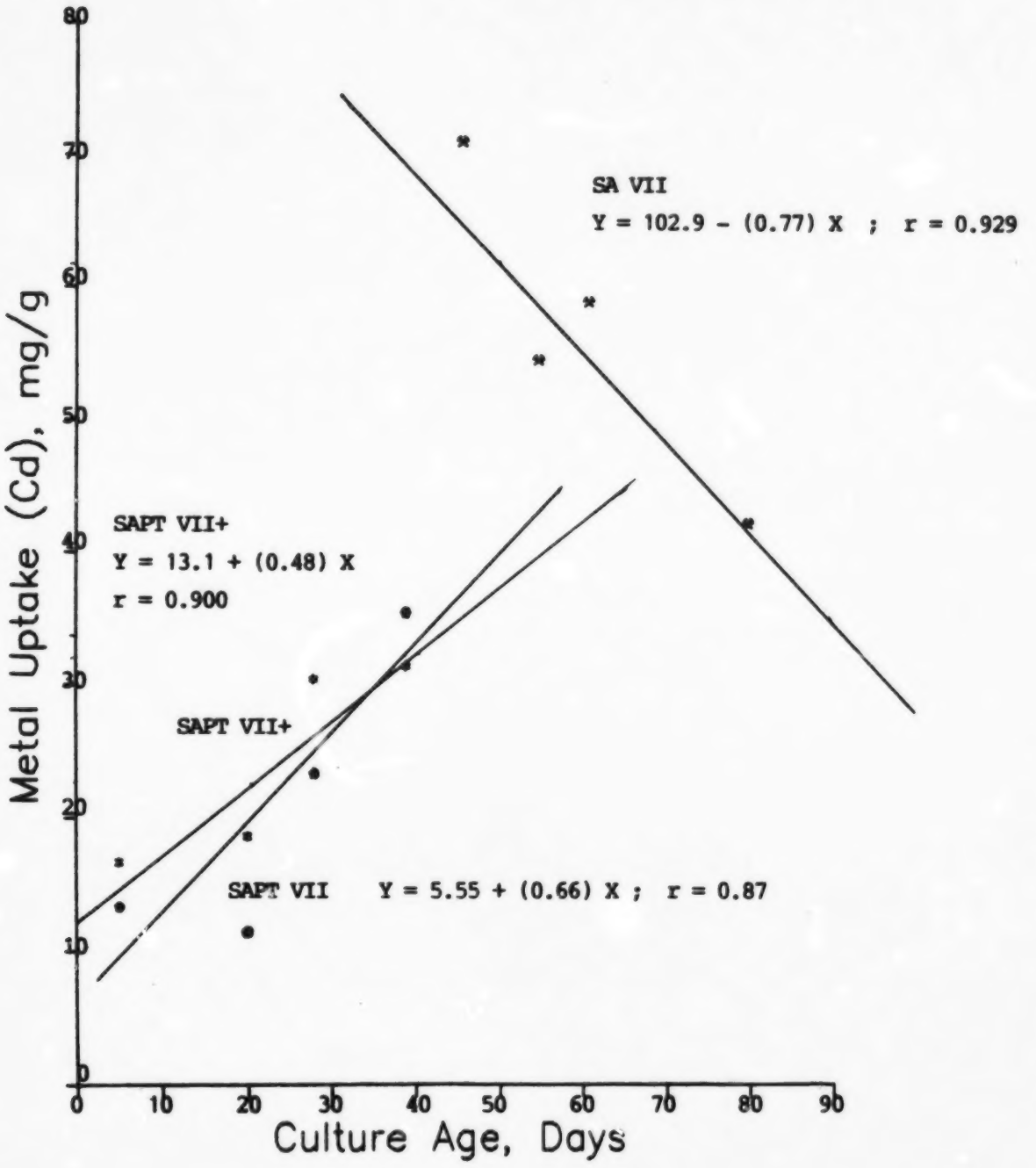


Fig.9. Effect of Culture Age on Cadmium Adsorption by Pretreated Algal Cultures; SAPT VII+, SAPT VII and SA VII.

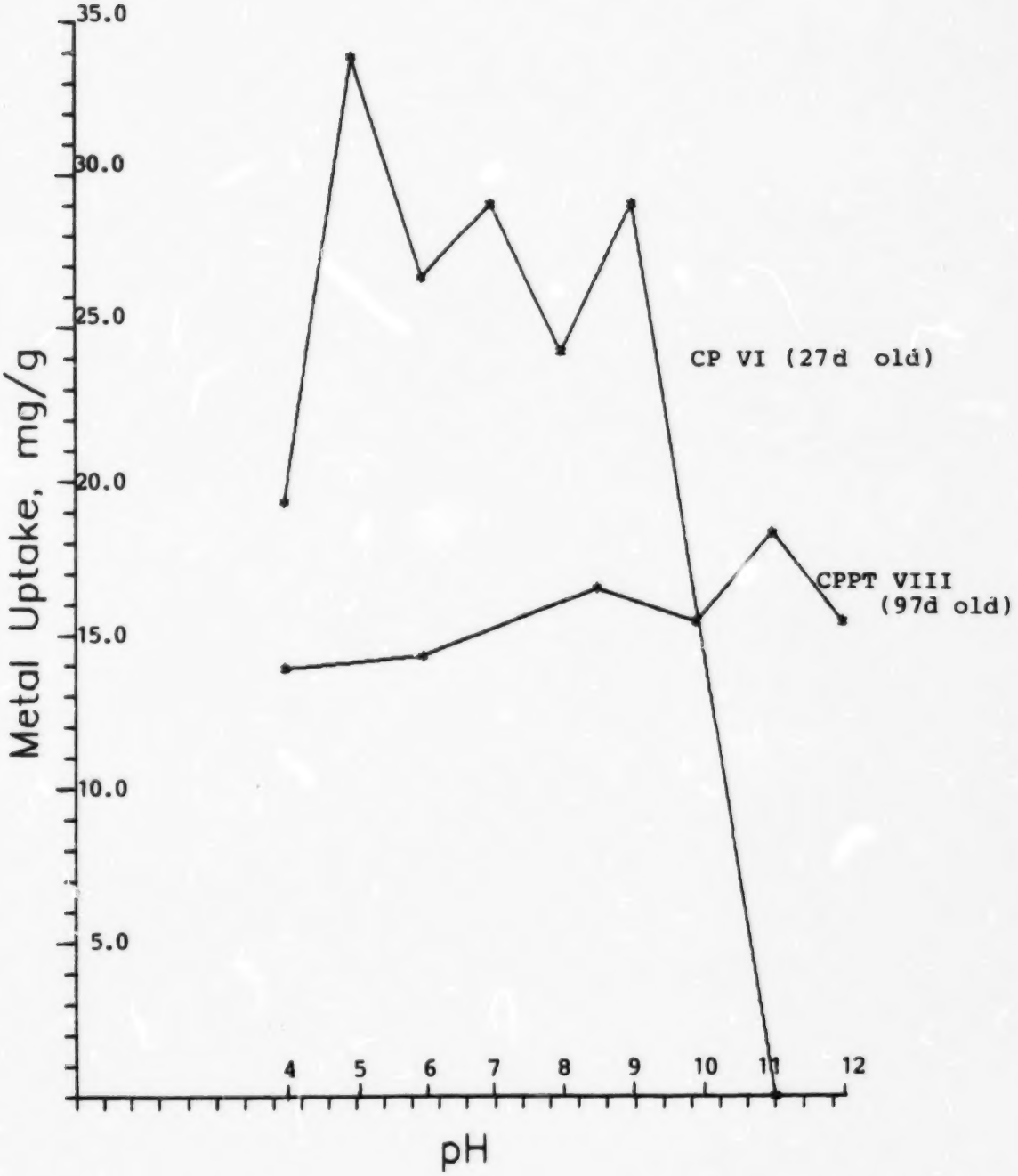


Fig.10 Adsorption of Cadmium by CP VI and Copper by CPPT VIII from a Metal Solution and Star Lake Gold Mine Effluent, Respectively at Various pH Levels.

the 97-day old CPPT VIII culture was more consistent in its repsonse with only a minor peak at pH 11. The adsorption at pH 12 was non-zero and close to its mean value at 15.6. The large alternating adsorption for the younger culture CP VI is indicative of the metabolic interaction in the metal removal process. At pH 11 the metal uptake was reduced by the possible rupture of the cell membrane. In a young cell, before the onset of the stationary phase or senescence, most of the metal uptake is governed by the metabolic processes which are susceptible to extreme pH variations. In an older cell, well past the stationary phase, most of the metal uptake is due to the adsorption processes operating in the cell wall or in the extracellular secretions. These are less prone to be affected by pH variations since the adsorbing compounds exhibit amphoteric behaviour and are active at either of the pH extremes. This was further demonstrated by the response of CPPT XIII and CPPT XV, aged 70 and 43 days respectively (Fig. 11). Although the uptake varied with the pH, the adsorption values at the pH extremes were still higher than the mean values. CPPT XIII was incubated with 0.5 mg/L of cadmium and 1.87 mg/L of clay whereas the culture CPPT XV was a pure culture.

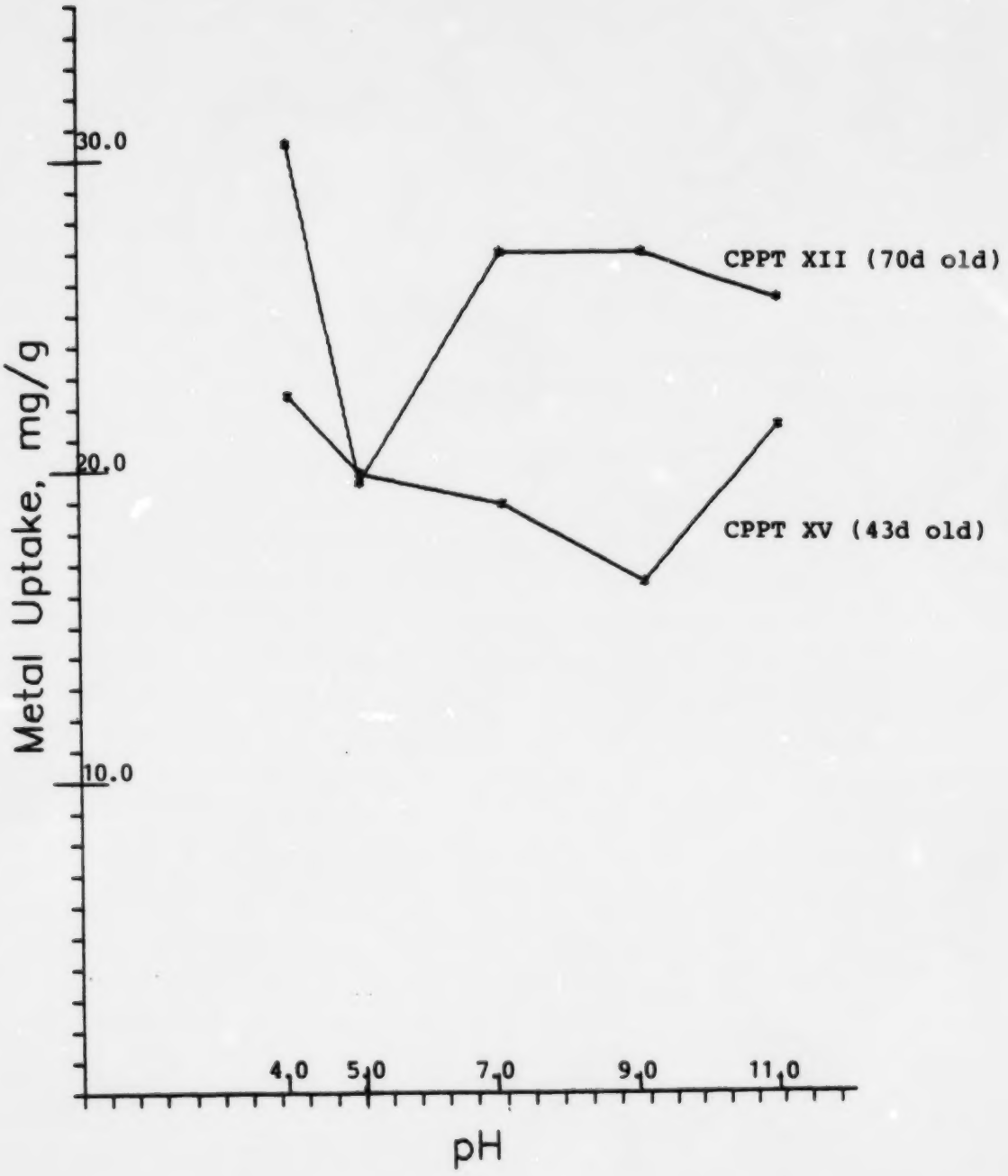


Fig.11 Adsorption of Copper from Star Lake Gold Mine Effluent by CPPT XIII and CPPT XV at Various pH Values.

CLAY-ALGAL COMPLEX:

Clay complexing experiments were conducted with CPPT and SAPT cultures and their copper adsorption was compared with the performance of pure clay mixtures. CPPT cultures consisted of CPPT XIA (27 days old), CPPT XIII (56 days Old), and CPPT VIII (100 days old). Clay-algal complexes contained 25 mg/L, 50 mg/L and 75 mg/L of bentonite clay and were mixed with Star Lake Effluent (SLE) for four minutes before filtering for analysis. Adsorption was evaluated at three pH values, 4, 7 and 9. The initial copper concentration in SLE was 20.0 mg/L. Clay concentration in the CPPT VIII complex was increased to 310 mg/L, 460 mg/L and 570 mg/L in separate runs.

Fig. 12 shows the data obtained plotted as copper uptake (adsorbed copper in mg per g of total adsorbent, mg/g) as a function of per cent clay content in the adsorbent. The data fell clearly into three distinct groups according to the pH. At pH7 the metal adsorption was insensitive to the variation in the clay content. However, at pH 9 the adsorption increased much more rapidly than at pH4. Similar experiments with SAPT IX produced opposite results. Copper adsorption decreased with increasing clay content. Experiments with pure clay at various concentrations resulted in similar inverse relationship with the clay content as the adsorbent.

Regression equations were obtained to establish the functional relationship between copper adsorption and the clay content. Complexes involving CPPT cultures produced a greater copper adsorption than SAPT complex and pure clay. This can be explained on the basis of competition by clay for adsorption sites on the algae reducing the chances for copper adsorption. It is also important to note that addition of clay to algae increases the total adsorbent mass more than it increases the total metal adsorption.

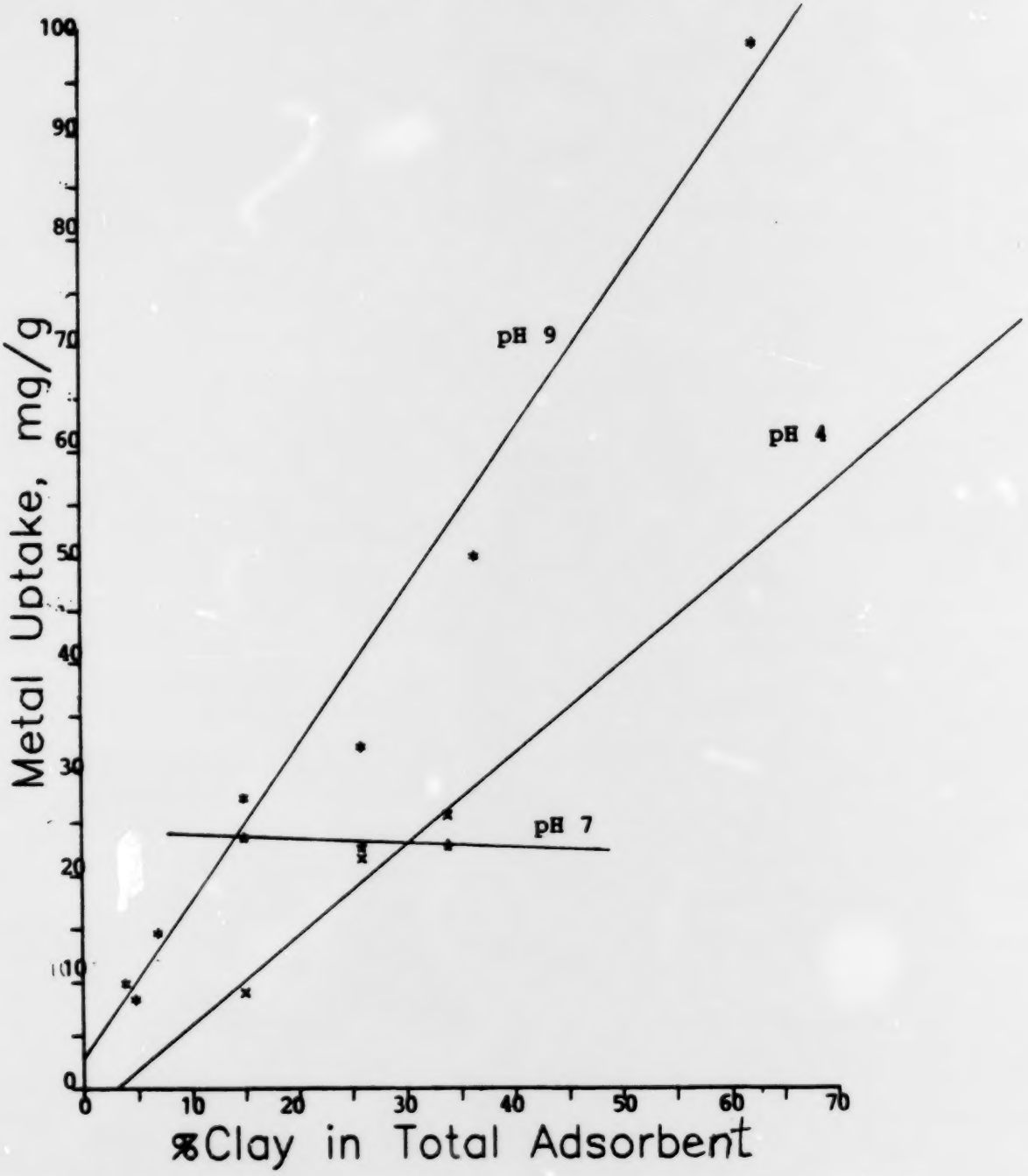


FIG. 12. Effect of % Clay Content in the Total Adsorbent on the Adsorption of Copper by CPPT XIA, CPPT VIII and CPPT XIII from the Star Lake Gold Mine Effluent.

This would lower the (mg/g) values for higher clay content. Regression data are presented in the following Tables.

Table. 7

Copper Uptake [mg/g] and % Clay in the Total Adsorbent

GROUP	pH	EQUATION	CORRELATION COEFFICIENT
CPPT CULTURES	9	$[mg/g] = (2.8)\ [\% \text{ Clay}]^{0.82}$	r = 0.984
	4	$[mg/g] = (0.3)\ [\% \text{ Clay}]^{1.31}$	r = 0.985
SAPT IX	9	$[mg/g] = (11.4)\ [\% \text{ Clay}]^{-0.24}$	r = 0.957

Further, regression equations relating copper uptake [mg/g] and clay content in mg were obtained which shed more light on the observed differences between CPPT cultures and SAPT cultures. Increasing the clay content reduced the copper uptake in pure clay and SAPT-clay complex, whereas it increased the uptake in CPPT-clay complexes. The following Table shows the regression relations obtained.

Table.8 Copper Uptake [mg/g] and Clay Content (mg)

GROUP	pH	EQUATION	CORRELATION COEFFICIENT
Pure Clay	‡	$[mg/g] = (82.4)\ [\text{clay}]^{-0.35}$	r = 0.999
Pure Clay	*	$[mg/g] = (3.32)\ [\text{Clay}]^{-0.21}$	r = 0.866
SAPT IX	9 +	$[mg/g] = (5.5)\ [\text{Clay}]^{-0.49}$	r = 0.999
CPPT XIA XIII, VIII	9 **	$[mg/g] = (40.1)\ [\text{Clay}]^{1.03}$	r = 0.606
	4	$[mg/g] = (30.9)\ [\text{Clay}]^{0.97}$	r = 0.976

‡ Clay content 9 - 75 mg. * Clay content 1.8 - 7.2 mg.
+ Clay Content 1 - 4.3 mg. ** Clay Content 0.3 - 1.5 mg

It is clear from the regression equations relating [mg/g] and clay content in mg that contribution by clay to the available adsorption sites was less than its contribution to the total adsorbent mass. This was so in pure clay and SAPT-clay complex. On the other hand, clay was quite effective when complexed with CPPT cultures, where the adsorption increased rapidly with the clay content in the total mass.

The addition of clay to algae appeared to produce a two-fold effect; First, it could effectively compete for the adsorption sites and reduce the metal uptake. This appeared to be the case with Scenedesmus acutus. Second, clay could act as a buffer to diminish the effect of pH on the algal cells. The diiferences in the algal response due to the culture age could also be masked by the clay's buffering action. This appeared to be the case with Chlorella pyrenoidosa.

At lower pH values such as around 4 and 5 the competition from protons could reduce the adsorption sites normally available for metal ions at these pH values compared with the adsorption at higher pH values.

The data obtained can be further examined in terms of the following design parameters.

$[M_a/M_p].10^2$ = Per Cent Adsorption

$[M_a/A_w].10^3$ = [mg/g]

= Metal Uptake per gram of Adsorbent

$[M_p/A_w]$ = Metal Loading

= Metal Available for Adsorption per Unit Adsorbent Mass

M_a = Metal Adsorbed, mg

M_p = Metal Available for Adsorption, mg

A_w = Adsorbent Mass, mg

These parameters are related through the following regression equations.

$$[M_a/M_p].10^2 = a_1[M_p/A_w]^{b_1}$$

[Per Cent Adsorption as a function of the Metal Laoding]

$$[M_a/A_w].10^3 = [mg/g] = a_2[M_p/A_w]^{b_2}$$

[Metal Adsorbed per gram of Adsorbent as a function of the Metal Loading]

a_1, a_2, b_1, and b_2 are regression constants.

Data were grouped into three categories; algae with or without clay as a complex, algae grown in low-nitrogen and high-light environment and pure clay. Fig. shows the plots obtained. In the algal group, per cent adsorption increased with the metal loading. Both Low-N algae and pure clay showed a negative effect of metal loading on the per cent adsorption. The culture ages ranged from 17 days to 100 days but the effect of culture age appeared to have been suppressed by the presence of clay.

Statistical data pertaining to the plots in the Fig.13 have been summarized in Tables.9 and 10.

Concentration Factor (CF) can now be defined to assess the effectiveness of various pretreatment methods including the light, nutrient and incubation regimes as well as clay-algae complexes. Concentration Factor, defined as the ratio of metal concentration in the adsorbent to the ambient concentration in the medium, helps to identify the appropriate combination for enhanced metal uptake under various ambient levels.

Computed values for [mg/g] and CF for various algal batches are given in Table. 11.

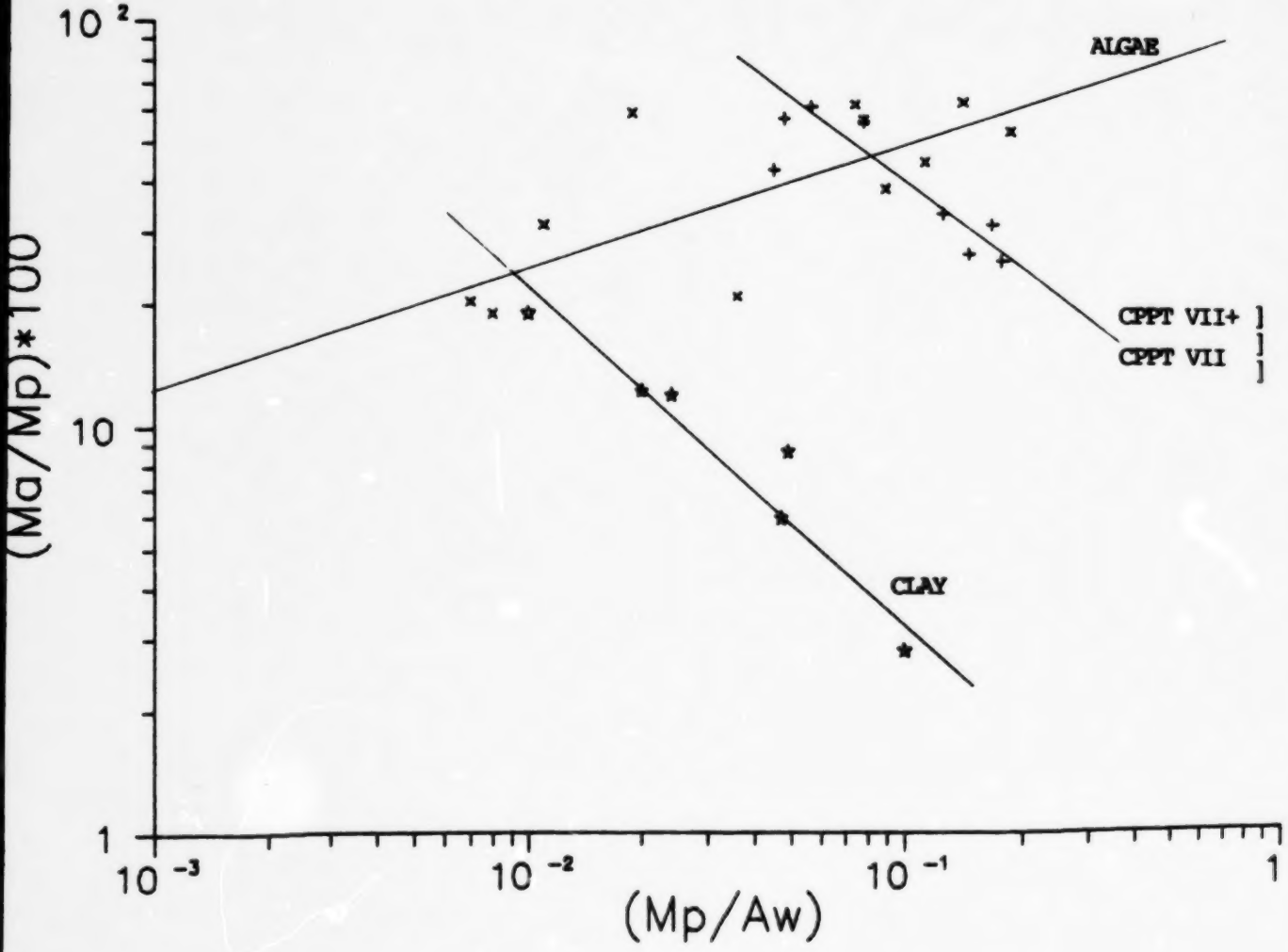


Fig. 13 Effect of Metal Loading (copper) on the Per Cent Adsorption from the Star Lake Gold Mine Effluent.

Table. 9 Per Cent Adsorption and Metal Loading

GROUP	EQUATION	CORRELATION COEFFICIENT
CPPT Algae & Algae - Clay Complex	$[M_a/M_p]x10^2 = [92.8]\,[M_p/A_w]^{0.28}$	r = 0.717
CPPT Algae without Clay	$[M_a/M_p]x10^2 = [96.4]\,[M_p/A_w]^{0.30}$	r = 0.680
Algae;Low-N CPPT VII+ CPPT VII	$[M_a/M_p]x10^2 = [11.1]\,[M_p/A_w]^{-0.53}$	r = 0.857
Pure Clay as adsorbent	$[M_a/M_p]x10^2 = [0.57]\,[M_p/A_w]^{-0.79}$	r = 0.955
SAPT IX	$[M_a/M_p]x10^2 = [0.80]\,[M_p/A_w]^{-0.12}$	r = 0.905

Table.10 Metal Uptake [mg/g] and Metal Loading

GROUP	EQUATION	CORRELATION COEFFICIENT
CPPT Algae & Algae - Clay Complex	$[M_a/A_w]x10^3 = [933.5]\,[M_p/A_w]^{1.28}$	r = 0.978
CPPT Algae without Clay	$[M_a/A_w]\,x10^3 = [971.9]\,[M_p/A_w]^{1.30}$	r = 0.972
Algae; Low -N CPPT VII+ CPPT VII	$[M_a/A_w]x10^3 = [105.9]\,[M_p/A_w]^{0.46}$	r = 0.825
Pure Clay as Adsorbent	$[M_a/A_w]x10^3 = [4.85]\,[M_p/A_w]^{0.15}$	r = 0.565
SAPT IX	$[M_a/A_w]x10^3 = [470.2]\,[M_p/A_w]^{0.89}$	r = 0.988

Metal uptake [mg/g] and Concentration Factor (CF) are both important parameters for consideration. Metal uptake is useful in designing metal recovery systems where it is neceassary to know the capacity of the adsorbing system in a dispersed state. Adsorption process, among other things, depends upon the ambient concentration and CF normalizes the response with respect to the concentration.

Table. 11 shows that high adsorption levels were achieved by CPPT VII, CPPT VII+, CPPT XIA w/clay. CPPT XIII CPPT XIII w/clay, CPPT XV and SA VII. Chlorella pyrenoidosa cultures were capable of enhanced metal adsorption under a variety of pretreatment conditions. They were also superior to Scenedesmus acutus in terms of growth rate, viability under high light, [mg/g] and CF. Adsorption levels as high as 98.5 mg/g were achieved. High adsorption rates were achieved with and without complexing, using low-nitrogen growth and optimum culture age and pH.

Table.11 Metal Uptake and Concentration Factor

BATCH	Metal Uptake Range	Mean	Concentration Factor	Mean
	mg/g	mg/g		
CP VI	19.3 — 33.8	27.0	171 - 354	252
CPPT VI	49.8		470	
CPPT VII	35.3 - 52.3	43.8	312 - 489	398
CPPT VII+	19.3 - 44.1	30.6	175 - 394	278
CPPT VIII	13.9 - 18.3	15.6	1236 - 1627	1390
CPPT IX	6.6		1146	
CPPT X	7.4		1278	
CPPT XI A-E	8.1 - 10.5	8.8	517 - 1741	1060
CPPT XI A w/clay	9.1 - 98.5	30.6	867 - 5868	2554
CPPT XIII	19.6 - 89.0	36.0	3077 - 5633	4236
CPPT XIII w/clay	50.5		3194	
CPPT XV	7.5 - 43.9	21.5	815 - 3209	2540
CPPT XV w/clay	45.7		2892	
SA VI	1.1 - 6.6	4.4	321 - 518	449
SA VII	4.2 - 70.7	56.4	396 - 656	513
SAPT VII	11.4 - 35.3	20.8	100 - 310	187
SAPT VII+	16.7 - 31.3	24.2	158 - 290	223
SAPT IX	0.5 - 10.1	4.9	312 - 1577	743
SAPT X	0.1 - 10.0	4.7	77 - 1643	693
SAPT XIIA	0.7 - 2.0	1.4	125 - 269	197

Concentration Factor: Ratio of the metal concentration in the algal cell to the metal concentration in the solution.

$$= \frac{\text{(microgram of metal per gram of cell weight)}}{\text{(milligrams of metal per litre of solution)}}$$

DESORPTION RUNS:

Desorption trials were conducted with CPPT XIII and CPPT XV, chosen for their high adsorption. Three experimental runs were completed, two with CPPT XIII and one with CPPT XV. Aliquots of algae were mixed with SLE, which varied in its initial copper concentration. After mixing for 20 minutes at pH4, algae were filtered and resuspended in distilled water adjusted to various pH values. To lower the pH 1N HCl was used and for higher pH Na_2CO_3 and NaOH were used. At each pH of interest the suspension was thoroughly mixed for 30 minutes and filtered. The filtrate was analysed for copper desorbed from the algae. The following Table gives the desorption data.

Table. 12 Desorption of Copper from Algae

	pH	Per Cent Desorption U	W	Y
	4	47.1	14.2	99.5
	5	100.0	24.3	82.5
	6.7	41.2		
	7			81.2
	7.1		31.3	
	9	38.2	48.2	
	9.3			89.0 *
				77.2 #
	11	100.0 *	59.9 *	60.2 *
				56.3 #
Culture Status		CPPT XIII Clay Complex	CPPT XIII Clay Complex	CPPT XV No Clay
Clay %		73	47	0
Age		72 days	79 days	58 days
SLE Cu		15.4 mg/L	7.38 mg/L	6.08 mg/L
[mg/g]		15.8	3.86	7.39

* Using NaOH
\# Using Na_2CO_3

Desorption data are plotted in Fig.14 . Sample Y continued to desorb almost at a uniform rate at all pH values. Sample U had two pH values where 100% desorption occurred, at pH5 and at pH11. With 73 % clay content it is possible that the algal response was suppressed by the presence of clay. The 100% desorption at pH5 and pH11 could be explained by the competition from protons and the rupture of algal membrane, respectively. However, the latter phenomenon was not noticed in earlier adsorption experiments. Sample Y, which was a pure algal culture, showed no such trends at these pH values. The CF values for the desorption runs were 1026 for U, 523 for W and 1220 for Y. Mean desorption percentages were 65.3 for U, 35.6 for W and 82.5 for Y. The data showed that the higher the CF value the greater was the desorption percentage. This can be explained on the basis of existence of a partition coefficient of adsorption between the algal and clay fractions. As adsorption begins to take place in a clay-algal complex, the saturation demand by each component is filled in a ratio equal to the ratio of saturation values. In the samples U and W, the clay-to-algae ratios were 2.7 and 0.89, respectively. Sample U had more clay to give it the characteristics of clay in terms of its response to pH. The complete desorption at pH 5 and 11 typical of clay. The sample W, with less than 48% of clay followed the algal adsorption trend. At low [mg/g] and CF values, the sample had not reached its maximum adsorption status.

Clay begins to dominate the adsorption behaviour of clay-algal complex at higher levels of clay content. Clay is most useful in the complex to enhance the metal adsorption at optimum levels determined by the culture age and algal species. However, SAPT cultures were quite unlike the CPPT cultures and reduced their adsorption capacity when complexed with clay.

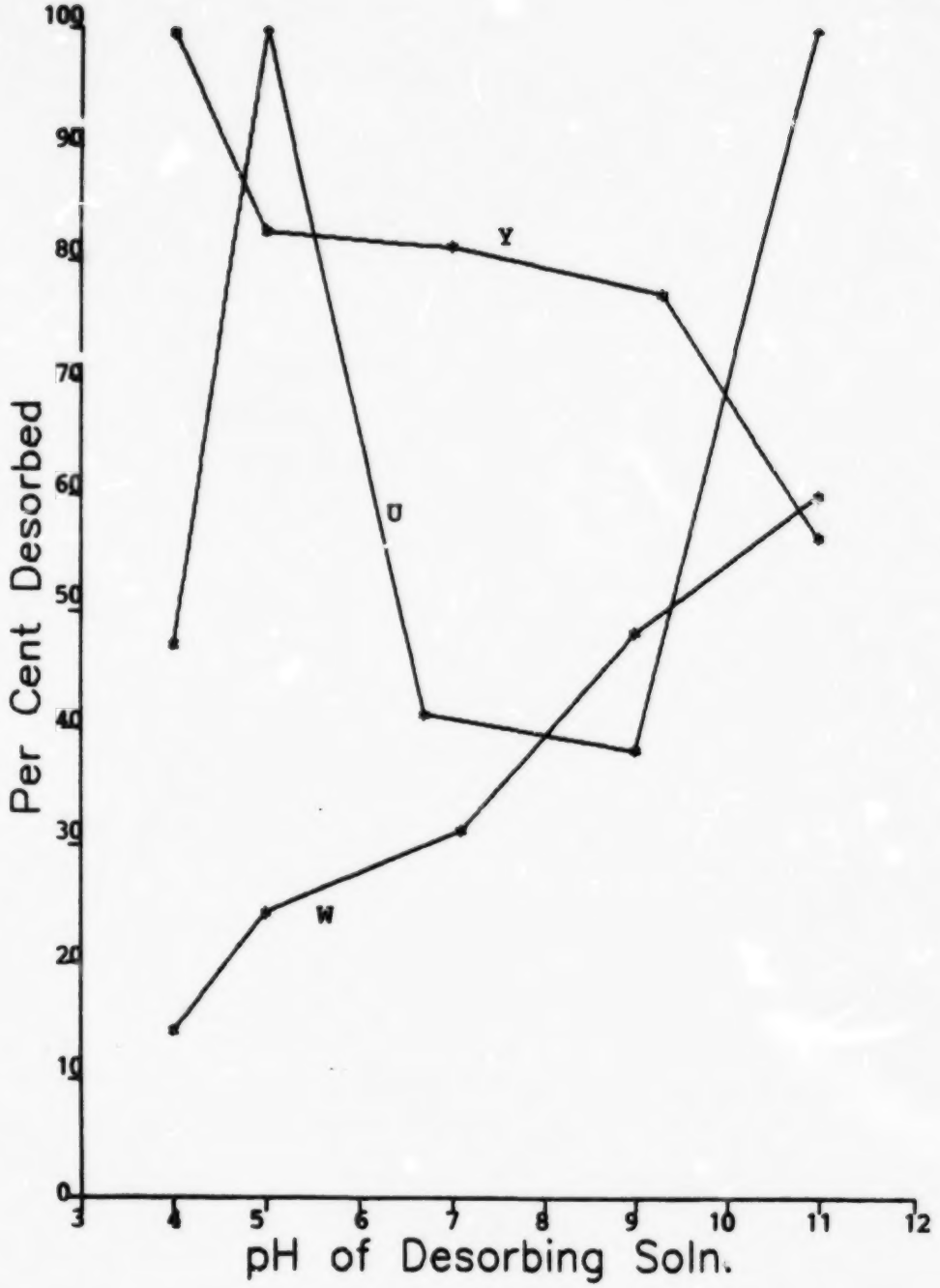


Fig.14 Effect of the pH of the Desorbing Solution on the Per Cent Copper Desorbed. (Star Lake Gold Mine Effluent)

On the other hand, CPPT cultures greatly enhanced their capacity for adsorption when complexed with clay. Obviously this was brought about by the production of extracellular organic compounds by Chlorella pyrenoidosa which were absent in Scenedesmus acutus.

The laboratory data are limited for providing a definitive explanation of the observed adsorption and desorption processes. However, the following observations can be made based on the knowledge of algal behaviour and published data.

1. Significant adsorption occurs in cultures older than their stationary periods. The metabolic uptake is greatly reduced during this period and any metal removal is entirely due to the increased adsorption.

2. Chlorella pyrenoidosa is capable of producing organic compounds such as polysaccharides, glycolic acid and others which offer large number of adsorption sites. Scenedesmus acutus does not appear to have the similar physiological response.

3. Clay-algal complexes formed with S.acutus behave similar to clay and the algae's capacity for adsorption is greatly reduced. Clay-complexed C.pyrenoidosa significantly inceases its adsorption.

4. The increase in the adsorption of clay complex depends upon the nature of bonding between the clay particles and the algal intact cell and any extracellular compounds.

5. If the clay contribution to the overall mass is greater than its contributed adsorption, the complex becomes more inefficient. The adsorption potential of a clay complex can be maximized by selecting the optimum pretreatment conditions, algal species, culture age and clay concentration in the complex.

6. The selection of species and clay concentration should ensure that clay does not suppress the algal response but will enhance the adsorption potential. At optimum clay-algae concentration ratio, the complex will perform much better than clay or algae alone.

7. Adsorption runs should be conducted at the pH where both clay and algae exhibit maximum adsorption potential and metal recovery through desorption should be conducted at the pH where the adsorption potential is the lowest.

CONCLUSIONS

1. Chlorella pyrenoidosa is well suited as an algal adsorption system for adsorbing and recovering copper and cadmium from mining effluents. Scenedesmus acutus is a close second choice. Both have sustained growth under stressful conditions such as high light (16 kilo lux) and low-nitrogen (33% of normal requirement, 25 mg/L).

2. During the post-stationary phase of the growth, adsorption is significantly enhanced, presumably by the release of organic compounds from the senescing cells. This is substantially the case in Chlorella cultures and this mechanism does not appear to occur in Scenedesmus cultures.

3. Algal cultures continued beyond their post-stationary phase do not exhibit any effect of pH on their ability to adsorb copper and cadmium. They are also more suited to be complexed with clay.

4. Clay-algal complexes are far more efficient than cadmium or clay incubated algae. No attempts were made to determine if Metallothionein type binding proteins were secreted by the algae under the various growth conditions.

5. Culture age and the clay concentration in the clay-algal complex are the two most important parameters which must be optimized to produce maximum metal adsorption.

6. Clay complexes formed with Chlorella cultures grown in normal and high nitrogen media produced increased metal uptake at higher metal loading. Similarly, Chlorella cultures without clay performed better than pure clay or Scenedesmus cultures.

7. Older algal cultures desorb uniformly at all pH values. Higher clay content in the complex suppresses the true algal response and produces a clay-like desorption pattern with pH.

8. Desorption rates as high as 100% can be achieved with a proper mix of algae and clay concentrations. Conducting the adsorption at the pH where the adsorption is maximum and desorbing at the lowest-adsorption pH would ensure an efficient adsorption and recovery process.

9. Maximum copper uptake from the Star Lake Gold Mine effluent was 98.5 mg/g with a concentration factor of 5868. There is a very strong case for exploring algal and clay-algal adsorption systems for enhanced metal recovery from mining effluents.

10. Further research is recommended to focus on the development of optimum clay-algal mixes for a pilot scale operation.

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APPENDIX

BOLD'S BASAL MEDIUM

[Modified According to Dr. J.Acreman, Curator, UTCC]

MACRONUTRIENTS	STOCK CONCENTRATION (g/L)	DILUTION For Full Strength Medium
KH_2PO_4	17.50	10 mL/L
$CaCl_2.2H_2O$	2.50	10
$MgSO_4.7H_2O$	7.50	10
$NaNO_3$	25.00	10
K_2HPO_4	7.50	10
NaCl	2.50	10
MICRONUTRIENTS		
EDTA. 2Na	10.00	1 mL/L
KOH	6.20	1
$FeSO_4.7H_2O$	4.98	1
H_2SO_4 (Conc)	1.00 mL	1
Trace Metal Mix		
H_3BO_3	2.86	1
$MnCl_2.4H_2O$	1.81	1
$ZnSO_4.7H_2O$	0.222	1
$NaMoO_4.2H_2O$	0.390	1
$CuSO_4.5H_2O$	0.079	1
$Co(NO_3).6H_2O$	0.0494	1